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ARMY MEDICAL RESEARCH INST OF INFECTIOUS DISEASES FR--ETC F/G 6/5
EXPERIMENTAL 'COXIELLA BURNETTI' INFECTION OF GUINEA PIGS AND S--ETC(U)
JAN 78 G H SCOTT, G T BURGER, R A KISHIMOTO

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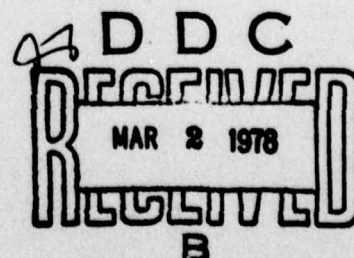
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EXPERIMENTAL COXIELLA BURNETII INFECTION OF GUINEA PIGS
AND SEVERAL INBRED STRAINS OF MICE^{1,2,3}

26 January 1978

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) EXPERIMENTAL COXIELLA BURNETII INFECTION OF GUINEA PIGS AND SEVERAL INBRED STRAINS OF MICE		5. TYPE OF REPORT & PERIOD COVERED
7. AUTHOR George H./Scott, Gary T./Burger Richard A./Kishimoto		6. PERFORMING ORG. REPORT NUMBER
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. Army Medical Research Institute of Infectious Diseases SGRD-UIA-E Fort Detrick, Frederick, Maryland 21701		8. CONTRACT OR GRANT NUMBER(s)
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research and Development Command, Office of the Surgeon General Department of the Army, Washington, DC 20314		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 3M762776A841 17/00/1 A841 00 012
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		12. REPORT DATE 26 Jan 78
		13. NUMBER OF PAGES 15 12/15 p.
		15. SECURITY CLASS. (of this report) UNCLASSIFIED
16. DISTRIBUTION STATEMENT (of this Report) Cleared for public release; distribution unlimited		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES Reprints bearing assigned AD number will be forwarded upon receipt. To be submitted for publication in Laboratory Animal Science.		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Inbred mice Rickettsia Q Fever		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The susceptibility of 5 inbred strains of mice and 2 strains of guinea pigs to <u>C. burnetii</u> infection by the respiratory, intraperitoneal, intratracheal, and intranasal routes was studied. The DBA/2J mice were more susceptible to infection and had higher mortality rates than the other strains of mice. Guinea pigs were more susceptible to infection than mice. Lesions observed in the infected animals were similar to that previously described in man and in experimentally infected animals.		

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Summary

The susceptibility of five inbred strains of mice and two strains of guinea pigs to Coxiella burnetii infection by the respiratory, intraperitoneal, intratracheal, and intranasal routes was studied. The DBA/2J mice were more susceptible to infection and had higher mortality rates than the other strains of mice. Guinea pigs were more susceptible to infection than mice. Lesions observed in the infected animals were similar to that previously described in man and in experimentally infected animals.

Inbred mice; Coxiella burnetii; rickettsia; Q fever

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FOOTNOTES

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² In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

³ The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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FOOTNOTE

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KEY WORDS

Inbred mice; Coxiella burnetii; rickettsiae; Q fever

Many domestic and laboratory animals can be infected with Coxiella burnetii (1-4), but mice and guinea pigs are considered by most investigators to be the experimental animals of choice. Both species are highly susceptible to infection, but death rarely occurs in either unless a high concentration of organisms is employed. Diagnosis of the disease or the evaluation of immunoprophylactic or therapeutic measures in experimental animal models is based upon febrile response, serological conversion, histopathological examination, or detection of rickettsiae in organs from these animals. A laboratory animal which would succumb to moderate doses of the rickettsiae would greatly facilitate studies with this organism. The present study was designed to test the susceptibility of several inbred strains of mice and two strains of guinea pigs to C. burnetii infection by the respiratory and parenteral routes.

The Nine Mile strain of C. burnetii in phase I was obtained from the American Type Culture Collection. A working stock of rickettsiae in 50% infected yolk sac suspension was prepared as described by Berman et al. (5). The titer of the rickettsial suspension was estimated to be $10^{9.5}$ mouse median intraperitoneal infectious doses (MIPID₅₀) per milliliter.

Outbred Hartley⁵ and Moen-Chase⁶ strains of guinea pigs of both sexes, outbred white Swiss-ICR female mice⁷ and five genetically standardized strains of inbred female mice designated as DBA/1J, DBA/2J, C57BL/6J, Balb/CJ and AKR/J⁸ were examined. All animals were 6-7 weeks old when tested.

Animals were exposed to either 10^5 MIPID₅₀ of rickettsiae in small-particle aerosols or were given between 10^5 and 10^8 MIPID₅₀ of

FOOTNOTES

- 5 Obtained from Buckberg Laboratory, Tompkin Cove, NY.
- 6 Obtained from CAMM Research Institute, Wayne, NJ.
- 7 Obtained from Charles River Breeding Laboratories, Wilmington, MA.
- 8 Obtained from Jackson Laboratory, Bar Harbor, MA.

rickettsiae intranasally (i.n.), intratracheally (i.t.), or intraperitoneally (i.p.). Control animals were given sterile 50% yolk sac suspension by either the i.n. or aerosol routes. Small-particle aerosols (mass median diameter, 2 μ m) were generated from a suspension of C. burnetii by a Collison atomizer (6) into a dynamic aerosol tube transport apparatus (7). The presented dose was computed on the basis of the MIPID₅₀/liter of aerosol x minute respiratory volume of the animal x the duration (min) of exposure (8). Preliminary tests indicated that when the inoculum was given i.n., no rickettsiae could be isolated from the lungs of guinea pigs. To assure that rickettsiae reached the lower respiratory tract, the trachea of anesthetized guinea pigs was surgically exposed and 0.2 ml of the diluted rickettsial suspension was inoculated directly. Mice and guinea pigs were inoculated i.p. with 0.1 ml and 0.2 ml of the diluted organisms, respectively. All animals were observed daily for overt signs of illness and death.

Blood was collected from surviving animals after 28 days and tested for anti-C. burnetii antibodies by the indirect immunofluorescent antibody (IFA) technique (9).

Prior to infection and at selected intervals after infection, randomly selected animals from each group were necropsied and various tissues fixed in buffered 10% formalin. Tissues were sectioned at 5-6 μ m and then stained with hematoxylin and eosin for histologic examination.

Following a 10^6 or 10^8 MIPID₅₀ challenge, illness was apparent in all strains of mice and guinea pigs. Animals developed clinical signs such as roughened fur, lethargy, excessive thirst, and coryza 3-7 days postexposure, and these signs usually persisted 4-7 days.

In many mice, infection was still evident at 28 days at which time the experiment was terminated. Only 2-11% of the white Swiss-ICR mice died, depending on the challenge route, and illness was observed only in the group that received the highest dose (10^8 MIPID₅₀). All inbred strains were more susceptible than the outbred Swiss-ICR mice as indicated by higher mortality rates (Table 1). Few deaths occurred following doses of less than 10^6 MIPID₅₀. Because the DBA/2J mice appeared most susceptible to i.n. exposure, their susceptibility to 10^5 MIPID₅₀ of airborne rickettsiae was compared to that of the outbred Swiss-ICR mice. The DBA/2J mice became overtly ill but did not die; no reaction was noted in the Swiss-ICR mice. This was the highest aerosol dose that could be administered practically with available equipment.

Both Hartley and Moen-Chase strain guinea pigs were more susceptible than mice to the lethal effects of rickettsiae administered by i.p., i.t., and aerosol routes. All guinea pigs of both strains died 8-12 days after exposure to 10^5 MIPID₅₀ in aerosol. Based on mortality rates, both strains of guinea pigs appeared equally sensitive to C. burnetii.

Lesions observed in the various strains of mice and guinea pigs were similar to those previously described in experimental animals and man (1-4, 10). Intrastitial pneumonia with mononuclear cell exudation into alveolar spaces occurred between 7-14 days in both mice and guinea pigs regardless of the route of infection. Following infection by the respiratory route, the frequency of pneumonia observed in inbred DBA/2J mice (13/15) was much greater than for outbred white Swiss-ICR mice (5/15); however, we could not detect

any histopathological differences in lung lesions that developed in the two strains. Essentially all of the guinea pigs developed pneumonia which persisted for 2-3 weeks regardless of the infection route. Other common pathologic findings in mice and guinea pigs were multifocal hepatitis, splenitis and lymphadenitis. Minimal lymphocytic myocarditis with lesions around vessels or in myocardial interstitial tissues was observed in some infected animals. All mice and guinea pigs developed anti-C. burnetii antibodies of 1:50 or greater 28 days following challenge regardless of the route of infection or the infecting dose.

In this study we have demonstrated that all strains of mice and guinea pigs tested were susceptible to infection with C. burnetii administered by various routes. Of the inbred strains, the DBA/2J mice were to be more susceptible to infection by the respiratory route and had a higher mortality rate. The severity of lesions did not appear to be related to dose.

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TABLE 1

Mortality in *C. burnetii* infected mice and guinea pigs

Strain	Number dead/total			
	Dose, MIPID ₅₀ and inoculation route			
	10 ⁸		10 ⁶	
	i.n.	i.p.	i.n.	i.p.
<u>Mice</u>				
DBA/1J	1/10	8/10	0/10	0/10
DBA/2J ^a	19/50	6/29	4/29	0/10
AKR/J ^a	1/40	12/40	0/40	4/40
C57BL/6J	0/20	14/20	1/20	3/20
Balb/CJ	1/20	16/20	0/20	2/20
Swiss-ICR ^a	1/55	6/55	0/40	1/39
	i.t.	i.p.	i.t.	i.p.
<u>Guinea pigs</u>				
Hartley	14/17	9/10	N.D. ^b	1/10
Moen-Chase	5/ 6	5/ 6	2/ 6	1/ 6

^a Data from 2-3 replicate experiments were pooled.^b Not done.